Mouse CD40 ELISA Kit（KT20435）

User Manual

For research use only. Not intended for diagnostic testing.

TABLE OF CONTENTS

I. Introduction……..…….2
II. Reagents……..……..2
III. Storage.…….…….. 3
IV. Additional Materials Required…….. .3
V. Reagent Preparation…… 3
VI. Assay Procedure…….....5
VII. Assay Procedure Summary……..... 7
VIII. Calculation of Results
A. Typical Data…..…….…….…7
B. Sensitivity…….…..……8
C. Recovery……...……..8
D. Linearity…….…....9
E. Reproducibility…….....9
IX. Specificity……...…. 9
X. Troubleshooting Guide.…….....10

I. INTRODUCTION

The Mouse CD40 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse CD40/TNFRSF5 in serum, plasma and cell culture supernatants. This assay employs an antibody specific for mouse CD40 coated on a 96-well plate. Standards and samples are pipetted into the wells and CD40 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse CD40 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of CD40 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

II. REAGENTS

1. CD40 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-mouse CD40.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution
3. Standards (Item C): 2 vials of recombinant mouse CD40.
4. Assay Diluent A (Item D): 30 ml diluent buffer, 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.
5. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Standard/Sample (cell culture medium) diluent.
6. Detection Antibody CD40 (Item F): 2 vial of biotinylated anti-mouse CD40 (each vial is enough to assay half microplate).
7. HRP-Streptavidin Concentrate (Item G): 200 µl 200x concentrated HRP-conjugated streptavidin.
8. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution.
9. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.

III. STORAGE

May be stored for up to 6 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at –80 °C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.
Note: the kit can be used within one year if the whole kit is stored at -20 °C. Avoid repeated freeze-thaw cycles.

IV. ADDITIONAL MATERIALS REQUIRED

1 Microplate reader capable of measuring absorbance at 450 nm.
2 Precision pipettes to deliver 2 µl to 1 ml volumes.
3 Adjustable 1-25 ml pipettes for reagent preparation.
4 100 ml and 1 liter graduated cylinders.
5 Absorbent paper.
6 Distilled or deionized water.
7 Log-log graph paper or computer and software for ELISA data analysis.
8 Tubes to prepare standard or sample dilutions.

V. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.

2. Sample dilution: Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of cell culture supernatants.

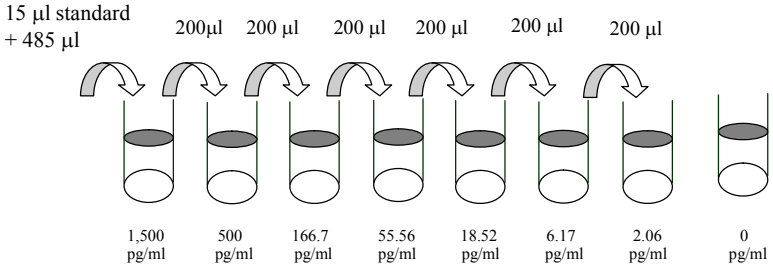
   Suggested dilution for normal serum/plasma: 3 fold*.

   * Please note that levels of the target protein may vary between different

specimens. Optimal dilution factors for each sample must be determined by the investigator.

3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

4. Preparation of standard: **Briefly spin the vial of Item C** and then add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernates) into Item C vial to prepare a 50 ng/ml standard. **Dissolve the powder thoroughly by a gentle mix.** Add 15 µl CD40 standard (50 ng/ml) from the vial of Item C, into a tube with 485 µl Assay Diluent A or 1x Assay Diluent B to prepare a 1,500 pg/ml standard solution. Pipette 400 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the 1,500 pg/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).



5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 200 fold with 1x Assay Diluent B.

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . A*dd 50 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent B to prepare a 200-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use).* Mix well.

VI. ASSAY PROCEDURE:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.

3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. Repeat the wash as in step 3.

8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.

⇩

2. Add 100 µl standard or sample to each well.
   Incubate 2.5 hours at room temperature or over night at 4°C.

⇩

3. Add 100 µl prepared biotin antibody to each well.
   Incubate 1 hour at room temperature.

⇩

4. Add 100 µl prepared Streptavidin solution.
   Incubate 45 minutes at room temperature.

⇩

5. Add 100 µl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.

⇩

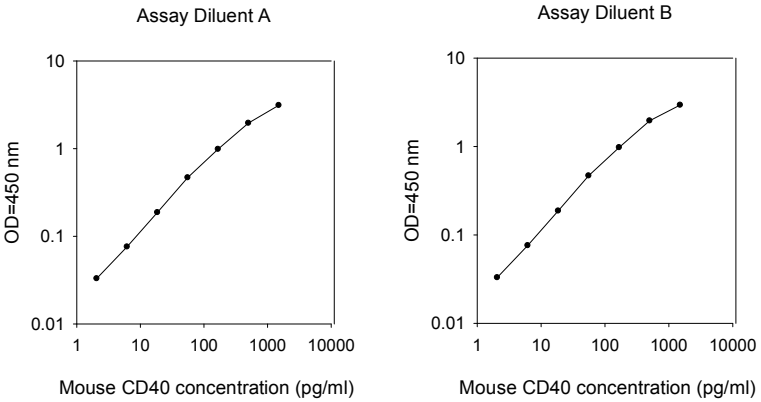
6. Add 50 µl Stop Solution to each well.
   Read at 450 nm immediately.

VIII. CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. TYPICAL DATA

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. SENSITIVITY

The minimum detectable dose of CD40 is typically less than 3 pg/ml.

C. RECOVERY

Recovery was determined by spiking mouse CD40 into mouse serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	77.26	71-87
Plasma	76.68	69-87
Cell culture media	84.23	74-93

D. LINEARITY

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	100.9	102.5	108.2
	Range (%)	91-110	93-110	97-117
1:4	Average % of Expected	104.9	102.0	108.0
	Range (%)	92-112	90-109	96-17

E. REPRODUCIBILITY

Intra-Assay: CV<10%
Inter-Assay: CV<12%

IX. SPECIFICITY

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin **,** Eotaxin-2, Fractalkine, GCSF, GM-CFS, IFN-γ, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 α, IL-1β, IL-2, IL-3, IL-3 Rb, IL-4, IL-5, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, MIG, MIP-1α, MIP-1γ, MIP-2, MIP-3β, MIP-3α, PF-4, P-Selectin, RANTES, SCF, SDF-1α, TARC, TCA-3, TECK, TIMP-1, TNF-α, TNF RI, TNF RII, TPO, VCAM-1, VEGF.

X. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1.Too brief incubation times	1. Ensure sufficient incubation time; assay procedure step 2 may change to over night
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the ELISA kit	1. Store your standard at<-20^{o}C after reconstitution, others at 4 ^{o}C. Keep substrate solution protected from light
	2. Stop solution	2. Stop solution should be added to each well before measure

Note:

This product is for research use only.

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